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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/087,714	02/28/2002	Daphna Havkin-Frenkel	DMCI-0099	7483

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EXAMINER	
COLLINS, CYNTHIA E	
ART UNIT	**PAPER NUMBER**
1638	

DATE MAILED: 12/20/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/087,714	HAVKIN-FRENKEL ET AL.	
	Examiner	Art Unit	
	Cynthia Collins	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 September 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *16,19-25 and 30-36* is/are pending in the application.

4a) Of the above claim(s) *30-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *16 and 19-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *1105*.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on September 8, 2005 has been entered.

Claims 1-15, 17-18 and 26-29 are cancelled.

Claims 32-36 are newly added.

Claims 16, 19-25 and 30-36 are pending.

Claims 30-31 are withdrawn.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

All previous objections and rejections not set forth below have been withdrawn.

Election/Restrictions

Newly submitted claims 32-36 are directed to an invention that is independent or distinct from the invention originally elected for the following reasons: the invention originally elected was directed to a method for improving production of vanillin in cultured *Vanilla planifolia* which comprises genetically engineering the cells to overproduce one or more enzymes associated with one or more steps of vanillin biosynthesis, wherein the enzyme is associated with chain shortening of p-coumaric acid to p-hydroxybenzaldehyde, and to genetically engineered

Vanilla planifolia cells and plants, whereas newly submitted claims 32-36 are directed to a method of expressing an enzyme having 4-hydroxybezaldehyde synthase activity in a cell of a plant, including plant cells from *Arabidopsis thaliana*, *Vanilla planifolia* and *Agrostis palustris*, and to genetically engineered *Vanilla planifolia* plants. Newly submitted claims 32-36 are directed to methods that result in the expression of a polypeptide, whereas the elected invention is directed to methods that result in the production of vanillin as consequence of the expression of a polypeptide. Newly submitted claims 32-36 also encompass methods wherein the enzyme is expressed in any plant cell of any plant species, including heterologous plant cells from *Arabidopsis thaliana* and *Agrostis palustris*, whereas the elected invention is limited to methods wherein the enzyme is expressed in the homologous species *Vanilla planifolia.*

Since applicant has received an action on the merits for the originally elected invention, claims 32-36 withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

Claim Rejections - 35 USC § 112

Claims 16 and 19-25 remain rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, for the reasons of record.

Applicants' arguments filed September 8, 2005 have been fully considered but they are not persuasive.

Applicants disagree with the Examiner's position that the nature of the rejection is due to the alleged unpredictability of the effect of overexpressing any gene on the production of vanillin, and that undue experimentation would be required to practice the claimed invention. Applicants note that the Office Action has not presented any objective evidence of record that overexpressing a rate-limiting enzyme would not be understood by the skilled artisan as a rational approach for improving metabolite production in plants, and one which would in fact be successful. (reply page 5)

Applicant's assertion that that the Office Action has not presented any objective evidence of record that overexpressing a rate-limiting enzyme would not be understood by the skilled artisan as a rational approach for improving metabolite production in plants, and one which would in fact be successful, is inapposite to the outstanding record. First, neither Applicants nor the Examiner nor the prior art of record have asserted or established that the 4-hydroxybenzaldehyde synthase of SEQ ID NO:2 is a rate-limiting enzyme in the biosynthesis of vanillin in *Vanilla planifolia.* In this regard the Examiner again notes that Applicant's own disclosure teaches that the hydroxylation of p-hydroxybenzyl alcohol to 3,4-dihydroxybenzaldehyde (proaldehyde) by a cytochrome P450 monooxygenase is believed to be the rate limiting step in vanillin biosynthesis (page 15 lines 2-6; page 16 lines 6-9), and that the conversion of 4-coumaric acid to 4-hydroxybenzaldehyde (by 4-hydroxybenzaldehyde synthase) is not considered to be the rate-limiting step in vanillin biosynthesis in cultured cells (page 20 lines 6-11). In this regard the Examiner also again notes that Havkin-Frenkel et al. (Food Technology, 1997, 51(11), 56-58, 61, Applicant's IDS) have likewise concluded that the hydroxylation of p-hydroxybenzyl alcohol (HBA) to pro-aldehyde (3,4-dihydroxybenzaldehyde)

is a limiting step in the vanillin biosynthetic pathway (page 57 column 1 second full paragraph to column 2 first paragraph).

Second, the Office Action does present objective evidence of record that overexpressing a rate-limiting enzyme in the biosynthesis of vanillin in *Vanilla planifolia* might not be successful. As set forth at page 7 of the Office action mailed May 6, 2004, Applicant's own disclosure teaches that in cultured cells much of the vanillin produced is reduced to vanillyl alcohol, which depletes the culture of accumulated vanillin (page 15 lines 10-13). In other words, even the overexpression of an enzyme that is known to be rate limiting for the biosynthesis of vanillin in *Vanilla planifolia* (or the overexpression of any enzyme that increases vanillin biosynthesis in *Vanilla planifolia*) might not produce the desired effect (improving vanillin production in *Vanilla planifolia*) if the reduction of vanillin produced to vanillyl alcohol depletes the culture of accumulated vanillin.

See also Walton N.J. et al.(Vanillin. Phytochemistry. 2003 Jul;63(5):505-15. Review), who teach that the metabolic engineering possibilities for enhancing vanillin production in *Vanilla* are not obvious (page 512 column 2). Walton N.J. et al. assert that there is no reason to believe that the activity of 4-hydroxybenzaldehyde synthase is limiting to the formation of vanillin β-D-glucoside. Walton N.J. et al. also suggest that further enhancement of vanillin production in green *Vanilla* pods may not be possible as their levels of vanillin β-D-glucoside are already high. Walton N.J. et al. additionally maintain that preventing the oxidation and reduction of vanillin formed as a consequence of metabolic engineering would not be trivial, because it could require the down-regulation of the enzyme activities responsible for vanillin oxidation and reduction, which enzymes would have to be identified and their genes isolated. Walton N.J. et al.

point out that it is also possible that these enzymes may perform other vital cellular functions, and that interference with such vital cellular functions as a consequence of trying to reduce vanillin oxidation and reduction could have deleterious effects.

Applicants also submit herewith references related to improving the production of metabolites by such techniques. Applicants note that it is generally understood in the art of plant metabolite production that carbon may be channeled or shunted into alterative or desired pathways by genetic manipulation, and that the references collectively teach that not only was it known in the art, before the time of filing, that for pathways at least as complex as that for vanillin, that the production of specific metabolites could be altered by such methods, it was even possible to increase the production of vanillin and related compounds by such methods (see e.g. Seibert et al. Plant Physiol. 1 12:81 1-8 19 (1996) and also Rasmussen and Dixon, Plant Cell 11: 1537-1551 (1999) (note Table 1). Applicants respectfully request reconsideration in view of the foregoing.

Applicants' arguments and the submitted references are inapposite to the claimed invention. The claimed invention is not directed to general methods of channeling or shunting carbon into alterative or desired pathways in plants by genetic manipulation. The claimed invention is directed to a specific method for improving the production of a specific compound (vanillin) in a particular plant species (*Vanilla planifolia*) by genetically engineering *Vanilla planifolia* to overproduce a specific type of enzyme (a 4-hydroxybenzaldehyde synthase of SEQ ID NO:2 obtained from a *Vanilla planifolia*) that acts on a specific substrate (4-coumaric acid) to produce a specific product (4-hydroxybenzaldehyde), whereas the submitted references are

directed to other types of methods which comprise genetically engineering other plant species to overproduce other types of enzymes which act on other types of substrates to produce other types of products.

Mayer M.J. et al. (Rerouting the plant phenylpropanoid pathway by expression of a novel bacterial enoyl-CoA hydratase/lyase enzyme function. Plant Cell. 2001 Jul;13(7):1669-82) teach genetically engineering tobacco to overproduce *Pseudomonas fluorescens* AN103 enoyl-CoA hydratase (crotonase) homolog (HCHL), an enzyme previously shown to convert 4-coumaroyl-CoA, caffeoyl-CoA, and feruloyl-CoA to the corresponding hydroxybenzaldehydes *in vitro*. Mayer M.J. et al. also teach that tobacco genetically engineered to overproduce *Pseudomonas fluorescens* AN103 enoyl-CoA hydratase (crotonase) homolog (HCHL) accumulated the glucosides and glucose esters of 4-hydroxybenzoic acid and vanillic acid and the glucosides of 4-hydroxybenzyl alcohol and vanillyl alcohol (page 1675 Figure 6; page 1677 Figure 7; page 1678 Table 3).

Siebert M. et al. (Genetic engineering of plant secondary metabolism. Accumulation of 4-hydroxybenzoate glucosides as a result of the expression of the bacterial ubiC gene in tobacco. Plant Physiol. 1996 Oct;112(2):811-9) teach genetically engineering tobacco to overproduce *Escherichia coli* chorismate pyruvatelyase, an enzyme not normally present in plants that converts chorismate into 4-hydroxybenzoate (4HB). Siebert M. et al. also teach that tobacco genetically engineered to overproduce *Escherichia coli* chorismate pyruvatelyase accumulated 4HB as beta-glucosides (page 816 Table 1).

Yu D.J. et al. (Metabolic engineering of medicinal plants: transgenic *Atropa belladonna* with an improved alkaloid composition. Proc Natl Acad Sci U S A. 1992 Dec 15;89(24):11799-

803) teach genetically engineering *Atropa belladonna* to overproduce *Hyoscyamus niger* Hyoscyamine 6 beta-hydroxylase (EC 1.14.11.11), an enzyme that catalyzes the oxidative reactions in the biosynthetic pathway leading from hyoscyamine to scopolamine. Yu D.J. et al. also teach that *Atropa belladonna* genetically engineered to overproduce *Hyoscyamus niger* Hyoscyamine 6 beta-hydroxylase accumulated scopolamine (page 11801 Table 1; page 11802 Figure 4).

Rasmussen S. et al. (Transgene-mediated and elicitor-induced perturbation of metabolic channeling at the entry point into the phenylpropanoid pathway Plant Cell. 1999 Aug;11(8):1537-52) teach genetically engineering tobacco cell cultures to overproduce a bean phenylalanine ammonia-lyase (PAL), an enzyme which converts L-phenylalanine to trans-cinnamic acid. Table 1 of Rasmussen S. et al. teaches that a vanillin derivative (Van-D) is produced in tobacco cell cultures genetically engineered to overproduce a bean phenylalanine ammonia-lyase (PAL) (page 1542).

The Examiner maintains that none of the submitted references provides guidance with respect to how to improve the production of vanillin in *Vanilla planifolia* by genetically engineering *Vanilla planifolia* to overproduce an enzyme having the amino acid sequence of SEQ ID NO:2 (a 4-hydroxybenzaldehyde synthase of SEQ ID NO:2 obtained from a *Vanilla planifolia*).

Such guidance is necessary because it is unpredictable whether the overproduction of an enzyme associated with chain shortening of p-coumaric acid to p-hydroxybenzaldehyde would improve the production of vanillin in *Vanilla planifolia*, as the chain shortening of p-coumaric acid to p-hydroxybenzaldehyde is but one of several steps required for vanillin biosynthesis.

Improvement of the production of vanillin in *Vanilla planifolia* cells by overexpression of p-hydroxybenzaldehyde synthase would depend not only upon the availability of sufficient p-coumaric acid substrate for the enzyme, but also on the downstream activity of other enzymes required to covert p-hydroxybenzaldehyde product into vanillin, as well as the activity of catabolic enzymes.

Given that multiple variables affect the production of vanillin in *Vanilla planifolia*, and given the lack of guidance in the disclosure and in the prior art, it would require undue experimentation for one skilled in the art to determine how to overproduce an enzyme having the amino acid sequence of SEQ ID NO:2 in a manner that would improve the production of vanillin in *Vanilla planifolia*, or in a manner that would produce a *Vanilla planifolia* cell which produces at least twice as much vanillin as a non-genetically engineered cell, as one skilled in the art would have to resort to trial and error experimentation in order to optimize, if possible, multiple variables in order to achieve the desired results.

Remarks

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Cynthia Collins whose telephone number is (571) 272-0794. The examiner can normally be reached on Monday-Friday 8:45 AM -5:15 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached on (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Cynthia Collins
Primary Examiner
Art Unit 1638

CC

Cynthia Collins
12/7/05